REMARKS

In the Office Action dated February 10, 2011, claims 22-24, 30-32, and 35-41 were pending, all of which were rejected. Claims 41 was objected to under 37 CFR §1.75 as allegedly being a substantial duplicate of claim 36. Claims 22, 24, 30, 37, 39 and 40 were objected to on formal grounds. Claims 31 and 35 were rejected on the ground of obviousness-type double patenting as allegedly unpatentable over various claims of U.S. Patent 7, 638,618 (hereafter "'618 patent". Claims 22-24, 30 and 36-41 were rejected on the ground of obviousness-type double patenting as allegedly unpatentable over various claims of the '618 patent in view of Dörschug (U.S. Patent 6,875,589). Claims 37-40 were rejected on the ground of obviousness-type double patenting as allegedly unpatentable over various claims of U.S. Patent 7,202,059 B2 (hereafter "'059 patent") in view of Dörschug and Schmid et al. (U.S. Patent 5,919,895). Claims 22-24, 30-32, 35-36 and 41 were rejected on the ground of obviousness-type double patenting as allegedly unpatentable over various claims of the '059 patent in view of Dörschug and Schmid, and further in view of Badziong et al. (U.S. Patent 5,866,371).

This Response addresses each of the Examiner's objections and rejections. Applicant therefore respectfully submits that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Claim Amendments

Claims 22, 24, and 30 have been amended in form, simply to maintain consistency with claim 23, as suggested by the Examiner.

Claim 37-41 have been canceled herein, without prejudice or disclaimer.

Upon entry of the foregoing amendments, claims 22-24, 30-32, and 35-36 will be pending.

Claim Objections

The objections to claim 41 and to claims 22, 24, 30, 37, 39-40 are obviated in view of the foregoing amendments. Withdrawal of the objections is respectfully requested.

Claim Rejections-Double Patenting Based on U.S. Patent 7,638,618 B2

With respect to the double patenting rejections of claims 22-24, 30-31 and 35-36, Applicants acknowledge that the rejections can be overcome by filing a terminal disclaimer over the commonly owned '618 patent. Applicants will defer responding to the rejections until after the Examiner determines the claimed subject matter to be otherwise allowable.

Claim Rejections-Double Patenting Based on U.S. Patent 7,202,059 B2

The rejection of claims 37-40 on the ground of obviousness-type double patenting over various claims of the '059 patent in view of Dörschug and Schmid, is moot in view of the cancellation of these claims.

The rejection of claims 22-24, 30-32, 35-36 and 41 over various claims of the '059 patent in view of Dörschug, Schmid and further in view of Badziong, is moot insofar as claim 41 is concerned in light of the cancellation thereof. The remaining claims, i.e., claims 22-24, 30-32 and 35-36 are not obvious over the identified claims of the '059 patent in view of Dörschug, Schmid and Badziong, for at least the following reasons.

The Examiner has acknowledged the differences between the allegedly conflicting claims. See Office Action, page 11, page 13 and page 14. However, in respect to the present claim limitation requiring a yeast ADH2 promoter and an alpha factor leader sequence not found in the claims of the '059 patent, the Examiner contends that Dörschug teaches a yeast expression vector encoding an alpha factor leader sequence, and Badziong teaches the use of a yeast ADH2

promoter for recombinant expression of miniproinsulin and hirudin in yeast (column2, line 16-20; column 3, line 10-12). In respect to the claim limitation requiring yeast as a host cell, also not found in the claims of the '059 patent, the Examiner states that Dörschug and Badziong both teach expression of a mini-proinsulin protein using a yeast host cell and expression vector. Therefore, the Examiner concludes that the present claims are obvious over the identified claims of the '059 patent in view of Dörschug, Schmid and Badziong.

Applicants respectfully submit that the alleged teaching in Dörschug for expression of a mini-proinsulin fusion protein in yeast is a fusion between the precursor sequence of mating factor alpha and mini-proinsulin, not a fusion protein containing hirudin as presently claimed. Additionally, in Badziong, the yeast ADH2 promoter is taught for recombinant expression of miniproinsulin and hirudin separately; i.e., there is no teaching for recombinant expression of a fusion between miniproinsulin and hirudin.

The Examiner has attempted to combine elements disclosed in separate prior art in establishing a *prima facie* obviousness case. With respect to a combination of prior art references, the Supreme Court states in its recent decision, *KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727 (2007), that an invention is not obvious if the combination of old elements is not a predictable use of these elements according to their established functions giving predictable results. See, e.g., *Id.*, at 13.

In the present case, it was not predictable at the time of the invention whether fusion proteins containing hirudin or hirudin derivatives at the N-terminus can be exported from yeasts in good yields. In fact, the finding in accordance with the present invention that the hirudin-miniinsulin fusion can be expressed and secreted in high yield was unexpected.

In support of Applicant's position, the Examiner's attention is respectfully directed to

the review publication by T. Kjeldsen (*Appl. Microbiol Biotechnol* 54: 277-286, 2000) (copy attached) which discusses the problems in secretory expression of insulin precursors from yeast at the time and various attempts to address the problems. Kjeldsen describes that the low yield of directly secreted insulin precursors was a particular problem at the time. *See* the abstract and page 280, left column, last paragraph of the article. In order to overcome this problem, a spacer peptide between the kexin2 cleavage site and the N -terminal amino acid of insulin B-chain was introduced, followed by a linear proinsulin sequence. *See* page 280, left and right columns; page 282, last paragraph; and page 283, left column, top two paragraphs. Apparently it was thought that the spacer peptide would enhance processing at the Lys Arg - dipetide by the processing enzyme kexin. However, as reported in Table 1 of the article, the yield of insulin precursor was still below 100 mg/L.

The Examiner's attention is further respectfully directed to the publication by Kjeldsen et al. (*J. of Biological Chemistry* 277: 18245-18248, 2002) (copy attached). This article describes that the yield of insulin precursor produced from yeast can be enhanced by 10-50% through a structure-based engineering which increases the folding stability, thereby improving secretion efficiency. The engineering involves creating a specific interaction between an aromatic amino acid in the C peptide and a phenol binding site in hydrophobic core of the molecule. The results reported in the article indicate that proper folding of the molecule is important for stability, which affects secretion efficiency.

While the art discloses that proinsulin can be produced using yeast as a host, the art does not teach or suggest how to design an insulin fusion protein which can be secreted by yeast in yields that are significantly higher than 100 mg/L. Around the priority date of the subject application (2001), the state of the art for recombinant insulin expression by yeast was to add a

short spacer peptide to the N- terminus of a linear proinsulin molecule that carried an optimized C- peptide spacer between B-chain and A-chain, as evidenced by Kjeldsen (2000). All fusion proteins described in the art at the time contained no additional cystein residues. The proinsulin precursor molecules appeared to be still poorly secreted, with a yield below 100 mg/L. It could have been due to some structural motifs within the insulin sequence that hindered secretion.

Although hirudin was known to be secreted in high yields in the gram range from yeast *H. polymorpha* (see, e.g., Weydemann et al., *Appl. Biotechnol* (1995) 44:377-385), it was not possible for the skilled artisan to reasonably predict at the time whether a hirudin-insulin fusion could be expressed and secreted in high yields similar to hirudin. It was expected that the secretion of the fusion protein could be hindered due to the intrinsic insulin motifs. In addition, hirudin contains 6 cysteines, hence the fusion of hirudin and insulin would lead to a polypeptide that contains 12 cysteine residues with the need to form 6 disulfide bridges in a correct manner. One would have expected that rnisfolding could occur, and misfolded polypeptides would be recognized by cellular control systems such as the ubiquitin system and degraded, resulting in lower yields. Additionally, the stability of the fusion protein could not have been predicted.

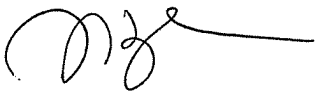
Surprisingly, the present inventor has found that the hirudin-mini-proinsulin fusion protein is secreted as well as the hirudin by itself, leading to equimolar high yields of proinsulin. In addition, folding of the fusion molecule is not impaired. Thus, unexpectedly, the fusion protein could be easily processed down to fully active, split insulin. The yields of insulin obtained are superior to that described in literature. Besides the advantage in yield, the approach provided by the present invention also provides the advantage of avoiding further modeling or structural engineering of insulin precursors. These superior and unexpected results achieved by the present invention are also described on page 3, paragraph [004] of the specification.

In light of the foregoing, the unpredictability in the art, and the unexpected results achieved by the present invention, Applicant respectfully submit that the presently claimed subject matter, directed to a process of making a hirudin-min-proinsulin fusion protein, is not obvious over the claims of the '059 patent, taken in view of Dörschug, Schmid and Badziong. Accordingly, the obvious-type double patenting rejection of claims 22-24, 30-32 and 35-36 based on the '059 patent, in view of Dörschug, Schmid and Badziong, is overcome.

Conclusion

It is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited

Respectfully submitted,



Xiaochun Zhu
Registration No. 56,311

Scully, Scott, Murphy & Presser, P.C.
400 Garden City Plaza- Suite 300
Garden City, NY 11530
(516) 742-4343
XZ:eb

Enc.: supporting references.